Case Report

Progesterone Receptor Gene Polymorphism Promoter Region +331G/A Increases Risk of Endometriosis

Polimorfisme Gen Reseptor Progesteron Regio Promoter +331G/A Meningkatkan Risiko Terjadinya Endometriosis

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Abstract

Objective: To identify relationship between progesterone receptor gene polymorphism promoter region +331G/A with the risk of endometriosis.

Method: An observational case-control study. Population are women with endometriosis and/or adenomyosis who have been performed laparotomy/laparoscopy at Obstetrics and Gynecology Department Dr. Mohammad Hoesin General Hospital Palembang, January-November 2013. Subjects fulfilled inclusion criteria, given informed consent and performed blood sampling continued by PCR-RFLP. Results were divided into A/A genotype (homozygote mutant), G/A (heterozygote mutant), and G/G (homozygote wild type). Data were analyzed by SPSS 21.0 version.

Result: PCR-RFLP results for+331G/A genotype were 26 (54.1%) in case group and 14 (26.4%) in control. +331A/A genotype was not found in both groups. There was significant increase risk of endometriosis in women carrying genotype +331G/A to those with genotype +331G/G with OR 3.29 ($p<0.05$).

Conclusion: Polymorphism on progesterone receptor gene +331G/A increases risk of endometriosis in Malay population in Indonesia. Compared to previous studies, there is interesting finding that polymorphic alels were found more commonly in our study.

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Keywords: endometriosis, polimorphism, progesterone reseptor, +331G/A

Abstrak

***Tujuan**: Mengetahui hubungan polimorfisme gen reseptor progesteron regio promoter +331G/A dengan risiko terjadinya endometriosis.*

***Metode**: Sebuah studi kasus kontrol observasional dengan populasi perempuan penderita endometriosis dan/atau adenomiosis yang dioperasi laparotomi/laparoskopi di Bagian Obstetrik dan Ginekologi RSUP Dr. Mohammad Hoesin Palembang dari Januari sampai November 2013. Subjek yang memenuhi kriteria inklusi, dilberikan informed consent selanjutnya dilakukan pengambilan darah dilanjutkan pemeriksaan PCR-RFLP. Hasil pemeriksaan dibagi menjadi genotif A/A (mutan heterozigot), G/A (mutan homozigot), dan G/G (wild type). Analisis data menggunakan program SPSS versi 21.0*

***Hasil**: I PCR-RFLP didapat genotif +331G/A sebanyak 26 (54,1%) pada kasus dan 14 (26,4%) pada kontrol. Pada kedua kelompok genotif +331A/A tidak ditemukan. Terdapat peningkatan risiko endometriosis pada perempuan dengan genotif +331G/A dibanding +331G/G dengan rasio odd 3,29 ($p<0,05$).*

***Kesimpulan**: Polimorfisme pada gen reseptor progesteron +331G/A meningkatkan risiko endometriosis pada populasi ras Melayu di Indonesia. Dibandingkan penelitian terdahulu, menarik mengetahui alel polimorfik ditemukan dalam jumlah yang banyak pada studi ini.*

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***Kata kunci**: endometriosis, polimorfisme, reseptor progesteron, +331G/A*

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INTRODUCTION

Endometriosis is a benign gynecologic condition characterized by presence of endometriotic tissue outside the uterine cavity. Chronic pelvic pain and infertility are two of its most common symptoms. Endometriosis is commonly found and affects about 5-10 % reproductive age women and more than 30% infertile women.[1-4]

Genetics study in endometriosis is attracting researchers in recent years. One of interest is polymorphism in progesterone receptor gene. Progesterone is a potent antagonist of estrogen-mediated-proliferation in endometrium and plays an important role in pathogenesis of endometriosis. Progesterone regulates this crusial function by its two isoform, A and B, which are expressed by one single gene located in chromosome 11q22-q23. PR-A is commonly found in endometrial stroma while PR-B in glandular which function by inducing cell apoptosis and secretoric change, repectively. PR-B acts as strong transcription activator to proges-

terone target genes, while PR-A is a dominat transrepressor of PR-B and ER-α activity anti-proliferative effect of progesterone depends on balance of these two isoforms.[3,5-8]

McDonnel, et al.[9] found the mechanism of which PR-A inhibits PR-B transcription activity by competitive inhibition to general transcription factor of mineralocorticoid receptor. While in non transcriptionally active cells, PR-A functions as glucocorticoid inhibitors, PR-B, and androgen receptor. Thus, repression of transcription activity of estrogen receptor depends on PR-A expression.[10-12]

Functional polymorphism at promoter region +331G/A of receptor progesterone gene (rs108 95068), by adding TATA box the transcription start site, promotes production PR-B relative to PR-A. Change in PR-A/PR-B ratio is believed to interfere stromal differentiation and lead to progesterone resistance. Thus, our hypothesis is that DNA polymorphism in progesterone receptor gene which interferes PR-A/PR-B ratio increases risk of endometriosis.[13]

METHODS

This was a case control study took place in Obstetrics and Gynecology Department and Microbiology Laboratory Dr. Mohammad Hoesin Hospital Palembang from Januari to November 2013.

Population were women with endometriosis and/or adenomyosis who have been performed laparotomy or laparoscopy in Obstetrics and Gynecology Department Dr. Mohammad Hoesin General Hospital Palembang.

Inclusion criteria were those in age 13-45 years and still have period. Endometriosis women who were pregnant, having ovarian carcinoma and myoma were excluded from the study. Controls were patients who undergone laparotomy or laparoscopy for other reasons and proven not having endometriosis and/or adenomyosis.

Blood samples were collected in EDTA tube and refrigerated in maximum temperature of 4°C until DNA extraction performed. Extraction was conducted using Chelex-100 method with phosphate buffer saline (PBS) pH 7.4; Safonin 0.5% in PBS; and Chelex 20% in ddH_2O pH 10.5.

DNA genome fragments were multiplied in vitro using oligonucleotide amplification primer pair to limit ampification area. We used 18pmol primer Forward 5'-CACTCATGGGATCTGAGAATC-3' and 18pmol reverse primer 5'-CACAAGTCCGGCACTT GAGT-3'. PCR was performed using lab cycler (Sensquest).

Polymorphism G/A in progesterone receptor promoter region +331 was detected from RFLP (restriction fragment length polymorphism) using retriction enzyme NlaIV.

PCR products with 330 bp length were digested by 1.0 unit NlaIV (isoschizomerBspLI, fermentas) with buffer reaction and incubated in 37°C temperature for 180 minutes. Then 5µl compound was poured into 2% polyacrylamide gel electrophoresis (PAGE) containing ethidium-bromide, then continued with electophoresis and visualized under ultra violet using Gel-Dov (BIO-RAD laboratories USA). Results were then analyzed using Quantity One software.

RESULTS

Within periods of January to November 2013, there were 101 subjects, 48 in case group and 53 in control group.

Mean age in case group of this study was 35.8 years. It corresponded with data showed that average age for endometriosis patients were 35-45 years. Patients in case group have significantly lower BMI than in control. More than half patients with endometriosis experienced infertility (52.1%), while only 7.5% of control group having infertility, p<0.001.

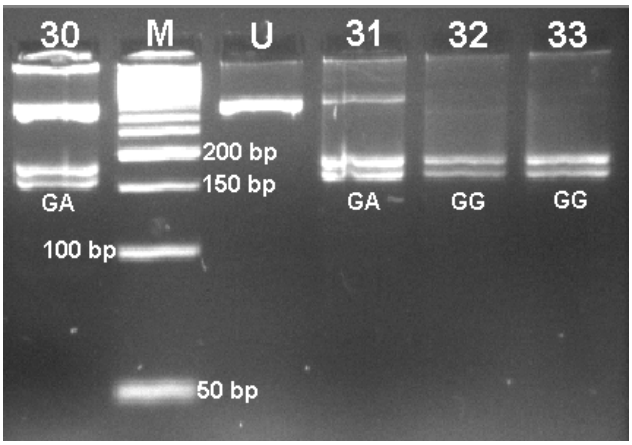

Figure 1. Visualization Result after pcr-RFLP

Table 1. Subject Sosiodemographic Characteristic

Characteristic	Case		Control		p
	Δ	%	Δ	%	
Mean age (year)	35,8		28,11		<0.001
Parity					
Nulliparous	33	68.8	24	9.4	
Primigravid	7	14.6	24	45.3	<0.001
Multigravid	8	16.7	24	45.3	
Mean BMI	22.96		25.47		0.003
Etnic group					
South Sumatra	37	77.1	39	73.6	
Jawa	8	16.7	10	18.9	
Minang	1	2.1	2	3.8	0.358
Cina	2	4.2	0	0	
Others	0	0	2	3.8	
Marital status					
Married	44	91.7	50	94.3	0.597
Unmarried	4	8.3	3	5.7	
Abortus history					
(+)	3	6.3	9	17.0	0.128
(-)	45	93.8	44	83.0	
Hormonal contraception					
(-)	36	75.0	41	77.4	0.255
(+)	12	25.0	12	22.6	
Infertility					
(+)	25	52.1	4	7.5	<0.001
(-)	23	47.9	49	92.5	
Smoking					
(+)	1	2.1	2	3.8	1.00
(-)	47	97.9	51	96.2	
Family history					
(+)	3	6.3	5	9.4	0.718
(-)	45	93.8	48	90.6	

Table 2. Distribution and Analysis of Progesterone Receptor Genotype

Genotype	Case	Control	Total
Genotype +331G/A	26 (54.1%)	14(26.4%)	40
Genotype +331G/G	22 (45.8%)	39 (73.5%)	61
Total	48	53	OR: 3.292 (95% CI 1.43-7.58), p<0,01

It can be said from the Table 2. that distribution of mutant heterozygote genotype was found more commonly in case group than control, p<0,01. There was increase risk of endometriosis in women carrying progesterone receptor gene promoter region +331G/A, with odd ratio 3.29 (95% CI.43-7.58). Homozigote mutant genotype (+331A/A) was not found in either group. Maybe because limitation of number of subjects studied.

We then further analyzed relationship between these two genotypes found in case groups with other variables such endometriosis level, infertility, and dysmenorhae. Correlation value between genotype +331G/A and endometriosis level based on ASRM was weak (0.27) with p>0.05 (Table 3). Genotype +331G/A had also weak correlation with dysmenorhae level, r 0.32 with p>0.05.[14]

Table 3. Distribution and Correlation Test between Genotypes and ASRM Endometriosis Level, and Genotypes with Dysmenorrhea Level in Case Group

Genotype	Endometriosis level					Dysmenorrhae level				
	I	II	III	IV	Total	No pain	Mild	Moderate	Severe	Total
+331 G/A	1 4.5%	6 27.3%	6 27.3%	9 40.9%	22	4 15.3%	6 23.0%	8 30.7%	8 30.7%	26
+331 G/G	0 0.0%	4 19.0%	3 14.3%	14 66.7%	21	1 4.5%	1 4.5%	10 45.5%	10 45.5%	22
Total* (%)	1	10	9	23	43 p= 0.33 r=0.27	5	2	23	18	48 p=0.14 r=0.32

**five adenomyosis only patients were not included*

DISCUSSION

There was significant age difference between case and control group. This can be explained because most subjects in control group were primiparous where as the others were mostly women who seeking for help after years of infertility. More than half of case group were infertile. This finding was along with the fact that endometriosis is one of commonest cause of infertility.[15]

Some previous researchers reported of specific group of women with tall and thin posture, short ovulatoar cycle, and heavy menstrual bleeding as to be "endometriosis phenotype". Women with normal BMI tend to have more regular cycle than those with higher BMI. From our findings, BMI average in case group was significantly lower than those in control group.

We also find in this study that presence of +331G/A genotype increased the risk of endometriosis with OR 3.29. While correlation of this genotype with the ASRM level and dysmenorrhea score was weak. Limitation of sample size and consecutive sampling methods were rationale of this result.[16]

Gentilini et al.[17] showed that this +331G/A polymorphic gene increased risk for deep infiltrating endometriosis with high invasive potential. It was assumed that PR-B, with a nontranscriptional mechanism, provokes cell development through interaction with estrogen receptor and stimulates Src/P21ras/ERK pathway. In investigating progesterone receptor on uterine myoma, Renner, et al.[14] found that +331G/A genotype was related to endometriosis incidence in myoma patients, which means this genotype was found more common in myoma patients having endometriosis.

De Vivo, et al.[18] stated that progesterone receptor gene polymorphism enhanced risk for endometrial cancer by increasing PR-B receptor expresssion.

This polymorphism was not related to cell ability to implant on ectopic sites but rather enabling cell invasiveness by altering PR-A and PR-B balance.

Different from those findings, Kaam, et al.[13] detected that +331A allele reduced risk of deep infiltrating endometriosis and adenomyosis. From our concideration, this result might be bias since allele +331A only found in 2 subjects. It was also reported from this study that ovarian ca patients did not show increase on +331A allele significantly.

Berchuck, et al.[11] reported lessen endometriosis risk on +331A variant allele in women participating in the study of ovarian carcinoma. Treloar, et al.[19] found there was no relationship between endometriosis risk with this variant.

Difference on study results were probably from different sample size, population and demography thus evoked variations in polymorphism pattern reported. In this study, conducted in Indonesia, we assumed to represented Malay population in Indonesia. On our best knowledge, this study was the first taken place in Indonesia. Previous researchers in different countries and etnic group reported various results. As we know, single nucleotide polymorphism is an inherited form and the incidence will be different among races.[20-24]

From this study we can conclude an interesting fact that polymorphism frequency in progesterone receptor gene promoter region +331G/A were found frequently in subjects, 54.1% in case group and 26.4% in control. Remembering that polymorphism is variable among groups and places, this study took place in Sumatera Selatan Indonesia revealed an attracting result.

Limitation of this study is that we did not analyzed progesterone receptor gene expression in endometriotic tissues. This gene expression examination is crucial to see how significant genotype polymorphism on tissue level, and to evaluate factors contributing to this.

CONCLUSION

Polymorphism on progesterone receptor gene promoter region +331G/A has significant relation with endometriosis. There is significant increase risk of endometriosis on Malay population in Indonesia having +331G/A gene compared to +331G/G gene. While endometriosis level based on ASRM as well as dysmenorrhea level are on weak correlation with this gene polymorphism.

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